GENERAL FEATURES OF THE EPIDERMIS IN ZEA MAYS[1]

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I. TECHNIQUES FOR STUDYING THE EPIDERMIS OF CEREALS AND GRASSES

In the plant kingdom the epidermis reaches its highest degree of differentiation in the Gramineae. Therefore it displays in this group a rich choice of specific characters which can be used in the genetic study of cereals as well as in the general taxonomy of the family (Prat, 1932). To obtain valuable results in this field it is necessary to make a careful examination of the epidermis on all the parts of the plant, by using a wide range of techniques and enlargements.

A. *Direct examination.*—

First, all the leaves and internodes from the base to the summit of the culm, as well as the floral bracts, must be examined with a dissecting binocular. All the features of the epidermis should be noticed, and at this stage of the work a preliminary distribution map of the most conspicuous elements can be drawn. However, this direct examination is never sufficient, some categories of cells requiring higher magnifications than others; hence the necessity of making cross-sections and peels.

B. *Peels.*—

The shortest way to obtain preparations of the epidermis is to peel off portions with forceps. However, this is possible only in the most favorable cases, while the technique of scraping described below serves under almost all conditions.

C. *Scraping.*—

Place on a glass slide the part to be studied: leaf, internode, etc., *the epidermis which is to be examined being face down.* Scrape away carefully with a scalpel all the overlying tissues, removing everything except the epidermis in question. Then turn the piece of epidermis upside down for microscopic examination. This operation is, in general, easy and rapid with fresh tissues. Dry material as, for instance, herbarium specimens, may be put in a softening medium of equal parts of glycerin, alcohol and water, for two or three days before dissection.

D. *Staining.*—

The pieces of epidermis can be observed immediately under the microscope. For a more detailed study of the cell walls the best technique is a double staining, using methylene blue and ruthenium red: (1) Put the tissue in a solution of

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methylene blue and alum for one-half minute; (2) wash it thoroughly; (3) place it in a watch glass in distilled water; then add a small quantity (less than a pin-head) of the powder of ruthenium red (solutions of this substance in water being unstable, it is necessary to prepare immediately at the time of use); let it remain for half an hour.

Cellulose cell walls will be colored a bright red, the intensity of color being chiefly in proportion to their content of pectic substances; sclerified cell walls will appear blue, suberous ones green. The progress of the ruthenium staining can be watched under the microscope until the desired intensity is reached. The preparation is then dehydrated rapidly in alcohol (70, 90, 100 per cent), put in xylol, and mounted in Canada balsam. Such slides can be kept indefinitely, though they decolorize slowly after some years. If ruthenium red, a rare and expensive substance, cannot be obtained, double staining by iodine green and alum carmine may be used successfully, though giving less brilliant colors.

E. *Observation.*—

The entire range of microscopic enlargements is useful for a study of the epidermis, from the lowest objectives for observing the general distribution of elements, up to immersion objectives for the tiniest cell details. All the resources of the condenser and of the diaphragm are needed to sharpen the contrasts between the elements and to accentuate their relief. For observing silica cells, hydrating mediums such as chloral lactophenol are useful in order to increase the difference in refraction indices between silica and neighboring tissues and to render those cells more conspicuous.

F. *Cross-sections.*—

Epidermic preparations obtained by peeling or scraping show the shape of the cells in vertical projection only. To recognize their other aspects it is necessary to examine also cross-sections of the whole organ, leaf or internode, both in transverse and longitudinal planes. These sections enable us also to observe the anatomical connections of epidermis cells with subjacent tissues. The best technique for staining them is again methylene blue and ruthenium red. After a careful microscopic examination of all epidermis preparations and cross-sections it is always necessary to examine again, in their living state, the entire organs of the plant with the dissecting binocular, in order to map the distribution of the elements precisely.

II. CATEGORIES OF ELEMENTS IN GRASS EPIDERMIS

Epidermis cells are regularly disposed in straight rows over almost the entire body surface of a grass. Some of them are elongated in the same direction as the organ on which they are borne (internode, leaf or bract), while others remain short; hence the classical distinction between "long cells" and "short cells." It is really better to distinguish "fundamental elements"—generally but not always elongated—and "differentiated elements."

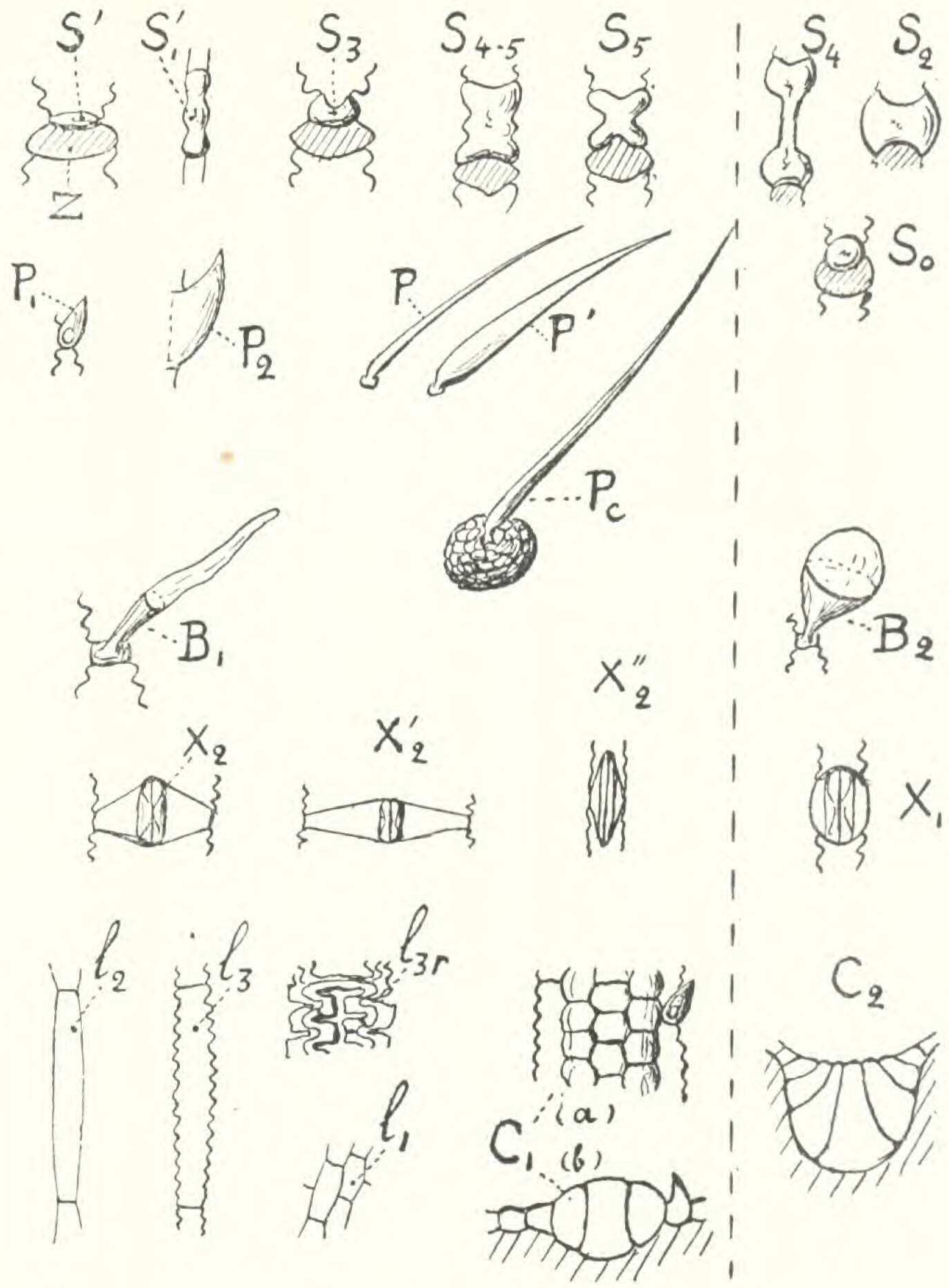

Fig. 1. Shapes of epidermic cells in grasses (structural characters). On the left of the broken vertical line, cells of *Zea Mays;* on the right, cells of other genera:

S, *silica cells:* S', S'_1, S_3, S_{4-5}, S_5, in *Zea;* S_4, in *Panicum* (shape widely distributed in panicoid tribes); S_2, in *Chloris* (shape general in chloridoid tribes); S_o, in *Hordeum* and festucoid tribes.

Z, *cork cells.*

Exodermic elements: P_1, small spicules; P_2, large spicules (profile); P, unicellular hair; P', swollen unicellular hair; P_c, cushion hair; B, bicellular hair; B_1, eu-panicoid shape; B_2, chloridoid shape.

X, *stomata:* X_2, lozengic shape present in *Zea,* with variants; X'_2, X''_2, X_1, ovoid shape (*Hordeum*).

l, *fundamental cells:* l_2, with straight walls; l_3, with undulated walls; l_{3r}, with thick and undulated walls; l_1, remaining short in transition zones.

C, *bulliform cells:* C_1, in *Zea* (a), vertical projection, (b) transverse section; C_2, in *Panicum* (transverse section).

It is worth noticing that only the first of these categories of cells is sensitive to auxins. At a given growth stage the length of a "long cell" in the meristematic base of an internode or of a leaf can be increased suddenly 200-fold in a lapse of several hours. During the same time the length of the adjacent "short cells" is only slightly modified. The body of these cells seems to escape the influence of growth hormones, probably on account of their precocious senescence; hence their shape is more constant and more interesting for displaying specific characters.

A. *Differentiated elements.*—

The differentiated elements fall into four great categories: (1) silica cells; (2) exodermic elements; (3) cork cells; (4) stomata.

1. The *silica cells* are precociously filled with a jelly of colloidal silica, which becomes a solid transparent block as the protoplasm dies. They may take the shape of halters, crosses, battle-axes, half-moons, etc., or be simply round or rod-shaped (fig. 1, *S*), these shapes being characteristic of sub-families and tribes of the Gramineae.

2. The *exodermic elements* include the differentiated cells or groups of cells which extend above the common level of the epidermis. Most of these are formed by a single cell: (1) *Unicellular hairs* (fig. 1, *P*); (2) Small or big *spicules* (P_1, P_2), which are present in all groups of the family. Others are formed by two or many cells, and they exist only in definite tribes: (3) *Bicellular hairs* (fig. 1, *B*), made up of two cells of different textures. The walls of the basal cell are strong and sclerified, those of the distal one, thin and cellulosic, giving an interesting example of unequal segregation of potentialities in the division of the mother cell; (4) *Cushion hairs* (fig. 1, *Pc*) consisting of big hairs formed by one cell but each arising in the middle of a hemispheric protuberance formed by a large number (20 or more) of small differentiated cells.

3. The *cork cells* (or suberous cells) (fig. 1, Z) do not by themselves exhibit characteristic shapes but are molded by neighboring elements. Their protoplasm dies early, being surrounded by impermeable walls of suberin. They offer an interesting case of cell polarity. When the mother cell of a differentiated cellular group divides, if a cork cell is produced it is always situated on the basal side of the group, i. e., toward the base of the organ. The other element, silicous or exodermic cell, is always apical.

4. The *stomata* (fig. 1, *X*) are formed by four cells, a special feature of the Gramineae, instead of two as is usual in other families. Their shape can be ovoid (X_1) or lozengic (X_2). Their distribution is interesting from the anatomical point of view on account of their connection with chlorenchyma, and from the ecological one. For example, in xerophytic species stomata are often absent on the outer face of the blade and localized on the inner face, being therefore enclosed in the leaf when it shrinks and rolls up into a tube during dry periods.

B. *Fundamental elements.*—

The fundamental cells always constitute the greatest part of the epidermic area. However, they are less interesting than the differentiated ones, their shapes being more uniform and exhibiting barely conspicuous specific characters. Their walls may be straight (fig. 1, l_2) or undulated in connection with small punctiform depressions in the outer wall (l_3). On strengthened regions these walls may become thick and deeply furrowed (l_{3r}). In certain species the outer wall may bear cuticular warts or hollow papillae. Cells of this category are generally much elongated. Their length may reach up to 300 times their width, but they may also, in certain areas, remain short, almost square (fig. 1, l), as in transition zones (nodes, sheath bases, blade bases). This is why the term of "long cells," usually applied to them, is a misnomer.

C. *Bulliform elements.*—

On the leaves of certain genera, chiefly on the inner face of the blade, regular stripes can be observed, consisting of "bulliform" cells. They are aquiferous, strongly turgescent cells, sometimes reaching a great volume by expanding perpendicular to the leaf surface (fig. 1, C_2). In vertical projection they are shorter than the neighboring fundamental cells (fig. 1, C_1a). They may be considered as a separate category, distinct from both fundamental and differentiated elements. By losing their water in dry air they function in the rolling of the leaf in some xerophytic species, hence the name "motor cells" which is sometimes applied to them.

III. DISTINCTIONS OF THE SUBFAMILIES OF GRASSES ACCORDING TO THEIR STRUCTURAL EPIDERMIC CHARACTERS

A. *Structural characters.*—

For an efficient utilization of epidermic features in taxonomy or genetics it is necessary, first, to recognize the respective values of all the characters which may be distinguished and to determine their order of subordination (Prat, 1933, 1936). Up to now we have referred only to the shapes of the epidermis cells and mentioned that they may differ from one group to another. In this way we find a first category—the *structural* characters. They are characters of the *first order*, i. e., they can be applied to distinguish the great subdivisions of the family: sub-families and tribes.

The first step in analyzing the epidermis of a Grass will be thus to determine *what shapes of cells are present.* In general, this is sufficient to reveal the subfamily, the tribe, sometimes the genus, and we can obtain this information on a tiny fragment of leaf some square millimeters in area; hence the importance of the method in certain practical researches, for instance, in tracing the origin of certain manufactured products.

B. *Subfamilies.*—

The subfamilies Bambusoideae and Panicoideae possess the most complicated shapes of silica cells (fig. 1, S_4, S_5) in the eu-panicoid tribes, (S_2) in the chloridoid ones. In the subfamily Festucoideae only the simplest types of silica cells (S, S_1) are present. In Bambusoideae and Panicoideae also there are bicellular hairs, threadlike (B_1) in the eu-panicoid tribes, and swollen (B_2) in the chloridoid. The cushion hairs (P_c), too, are correlated with the Panicoideae subfamily. The true Festucoideae never possess either bicellular or cushion hairs (Prat, 1936).

Thus we may distinguish the structural epidermic characters of the great subdivisions of the Grass family:

(1) Bambusoideae, primitive group including arborescent forms, with the most complex epidermis.

(2) Panicoideae, mostly a herbaceous group retaining complex shapes of epidermic cells but with a specialization appearing in two directions:

(a) *Eu-panicoid type* characterized by halter-shaped silica cells (S_4), threadlike bicellular hairs (B_1). Includes the tribes Paniceae, Andropogoneae, Maydeae.

(b) *Chloridoid type* characterized by silica cells molded in the shape of battle-axes with double edges (S_2) and by swollen bicellular hairs (B_2). Includes the tribes Chlorideae, Eragrosteae, Sporoboleae.

(3) *Festucoideae*, herbaceous subfamily showing a marked simplification of the epidermic cell shapes, absence of bicellular and cushion hairs, and only the simplest shapes of silica cells (S, S_1). This group includes the tribes Festuceae, Hordeae, Aveneae, Agrostideae.

These structural differences in the epidermis between the subfamilies are in perfect harmony with equally important anatomical and cytological differences which distinguish the same groups; for instance, the radial disposition of the chlorenchyma around the vascular bundles in the leaves of Panicoideae, but not in Festucoideae; the basic number of chromosomes, 7 in Festucoideae, 5 or 9 in Panicoideae (Hunter, 1934); the first green leaf of the seedling, narrow and vertical in Festucoideae, broad and extroverted in Panicoideae, etc. The geographic distributions, too, are different: Bambusoideae and most of the Panicoideae are localized in tropical regions; Festucoideae in temperate and cold countries.

The concordance between these diverse categories of characters—epidermic, anatomical, cytological, etc.—gives a practical basis for revising the systematics of the Gramineae in order to reach a more natural classification showing the real affinities of the genera (Prat, 1936), the present classifications still being artificial and inadequate in many parts.

IV. STRUCTURAL CHARACTERS OF THE EPIDERMIS OF *ZEA MAYS*

A. *Shapes of cells.*—

On the leaves of maize we found the following categories of epidermic cells (see fig. 1, left side):

(1)—silica cells of the types: S', S'_1, S_3, $S_{4\text{-}5}$, S_5.
(2)—small and big spicules: P_1, P_2.
(3)—thread-like bicellular hairs, B_1.
(4)—cushion hairs, P_c.
(5)—cork cells, Z.
(6)—quadricellular lozengic stomata, X_2.
(7)—fundamental cells, mostly with undulated walls, l_3, and also, on localized areas, the types l_{3r}, l_2 and l_1.
(8)—bulliform cells of the type C_1.

B. *Taxonomic position of Zea Mays.*—

This epidermic structure belongs strictly to the eu-panicoid type and agrees with the anatomical and morphological characters in placing the genus *Zea* in the subfamily Panicoideae, near the eu-panicoid tribes Andropogoneae and Paniceae. The recognized affinities of the Maydeae with the Andropogoneae are thus confirmed by the structural epidermic characters.

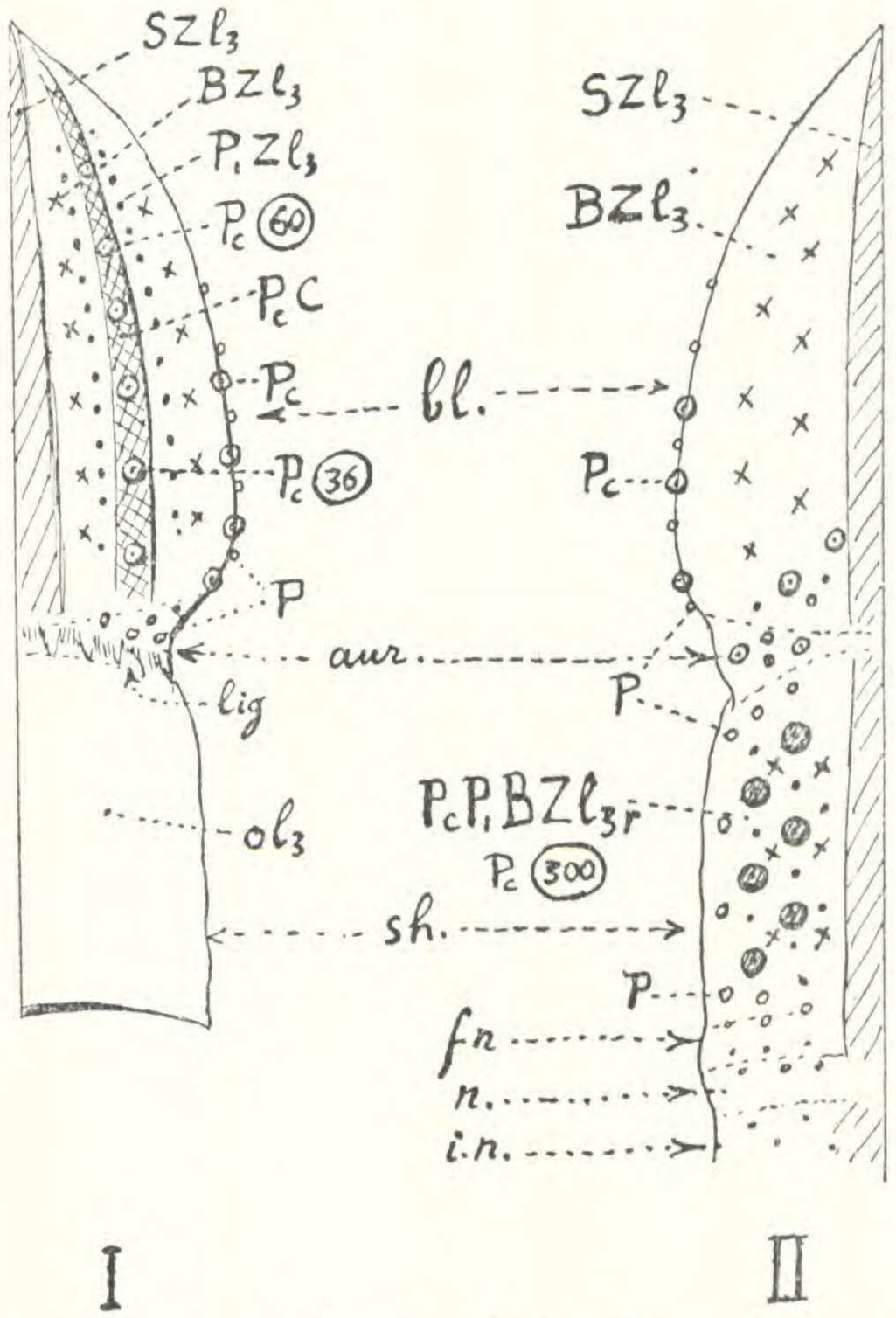

Fig. 2. Dermogram of the upper leaf, Mexican dent corn No. 1061 (distributive characters):

I, inner face of the leaf; II, outer face.

bl, blade; aur, auricular triangle; lig, ligula; sh, sheath; fn, false node; n, node; i.n., internode.

Letters representing the epidermic cells are the same as in fig. 1. Cushion hairs are figured by dots in circles (hatched when the cushion is colored red), ordinary hairs by circles, spicules (P_1) by dots, bicellular hairs (B) by crosses, bulliform stripes by cross-hatching, dense silica cells by hatching. Numbers in circles show the average density of cushion hairs per square centimeter.

V. DISTRIBUTIVE CHARACTERS; DERMOGRAMS OF CORN LEAVES

A. *Characters based on the distribution of epidermic cells.*—

The structural characters are not the only ones to consider in the epidermis of a Grass. The distribution of the different types of cells on all the organs of the plant (leaves, internodes, glumes, etc.) obeys precise rules and shows notable differences from one species to another, and within a species from one variety to another. The *distributive characters* thus constitute a second category of taxonomic importance. They are less fundamental but allow more delicate distinctions than the structural ones, as they provide a means of distinguishing smaller groups (species or varieties) instead of subfamilies and tribes. For this reason they are the most valuable for taxonomic or genetic work.

B. *Conception of dermograms.*—

The distributive characters can be shown in "dermograms," schematic maps figuring the position of the principal categories of epidermic cells. Figures 2 and 3 show examples of leaf dermograms for two varieties of Maize. The leaf represented must preferably be the upper one, just below the tassel, for this leaf displays to the utmost the specific characters. The leaves at the base of the culm are less differentiated, this fact being connected with the general law of histological gradation (Prat, 1934, 1945).

In figs. 2 and 3 only one-half of the leaf is represented, inner and outer face, and on this only one vein. The complete representation of all the veins with all their epidermic cover would give an infinitely complicated and useless diagram. For the need of simplification also, only the elements of interest for systematic comparisons are figured. For instance, stomata are not indicated on the scheme, their distributions being about the same in the varieties under comparison.

According to their size, spicules are figured by small or big dots (P_1 or P_2), unicellular hairs (P) by circles, bicellular hairs (B) by crosses, cushion hairs (P_c), by dots in circles. Regions where the differentiated elements are absent or consist only of stomata are left in white (ol); bulliform stripes (C) are represented by cross-hatching; regions with abundant silica cells (S) by hatching.

The precision of the dermogram can be increased by indicating the density of the most characteristic elements on different regions of the leaf. This density is easy to calculate by counting these elements in the field of a dissecting binocular for the biggest ones, such as cushion hairs, or in the field of a microscope for the smallest, such as bicellular hairs, and reducing to the unity of surface. On fig. 2 the numbers inscribed in circles indicate the local density of cushion hairs (P_c) per square centimeter.

C. *First example: Mexican Dent No. 1061.*—

The first example (fig. 2) is a small-seeded Mexican dent corn from El Capulin, bearing the number 1061 in the experimental cultures of Dr. E. G. Anderson, California Institute of Technology. The most striking feature of this

variety is the abundance of cushion hairs (P_c) on the entire outer face of the sheath (base of diagram II, fig. 2). Their density reaches here an average of 300 per square centimeter. On this area the cushion cells are colored a strong purple by an anthocyanin contained in their vacuole, and those cushions are conspicuous to the naked eye as small red dots. The hair itself is white, appearing under the microscope as a giant column with a constricted base, arising in the middle of the protuberance formed by the colored heap of dwarf cushion cells. The contrast between white hairs, purple cushions, and green surrounding tissues (chlorenchyma appearing through the uncolored flat epidermis) is striking when observed with the dissecting binocular, the more so as the cushion protuberances are strongly marked in this variety. The purple tint of the cushions appears only on the portions of sheaths exposed to the light.

On the outer face of the sheath the density of cushion hairs can reach up to 300 per square centimeter. We may notice also on the outer face small spicules (P_1), bicellular hairs (B), cork cells (Z) and, chiefly on the veins, silica cells (S). The fundamental cells present strongly undulated and thickened walls (l_{3r}). The inner face of the sheath (base of I, fig. 2) is covered by the simplest type of epidermis—only thin-walled fundamental cells (l_3) with few stomata.

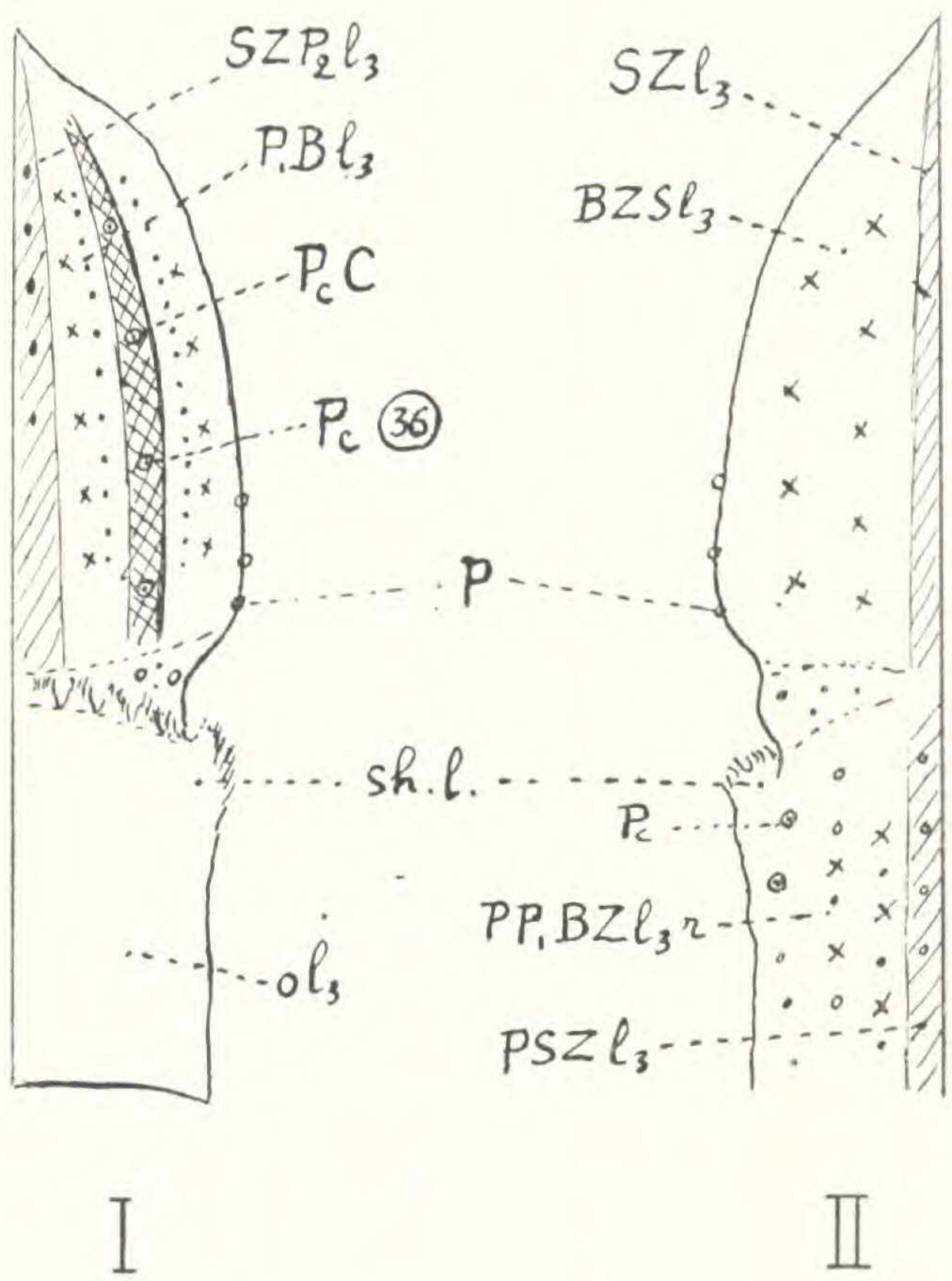

Fig. 3. Dermogram of the upper leaf, inbred yellow dent corn L317 (distributive characters):

Sh. l, sheath lobes; other conventions same as in fig. 2.

The inner face of the blade (summit of I, fig. 2) offers as its most striking feature conspicuous stripes of bulliform cells (C) located between the veins. On these stripes we observe from place to place big cushion hairs (P_c) analogous to those of the sheath, with the difference that their cushion is not colored purple though it is exposed to the light. On each side of a bulliform stripe is a dense row of small spicules (P_1). In addition, the inner face of the blade shows bicellular hairs (B_1), cork cells (Z), and chiefly on the ribs, silica cells (S). The fundamental cells (l_3) have undulated walls of medium thickness. In this variety the density of cushion hairs is lower on the inner face of the blade than on the outer face of the sheath; it can vary from 36 per square centimeter at the base of the blade up to 60 at the summit.

On the outer face of the blade (summit of II, fig. 2) we find no bulliform stripes, and only very rarely cushion hairs, localized at the base of the blade, mainly on the edges and on the auricular triangle. The exodermic elements are represented chiefly by bicellular hairs, spicules being scarce. The difference between the two faces of the blade can be recognized simply by feeling them with the finger tip, the inner face being minutely scabrous on account of its numerous small spicules (P_1), the outer face perfectly smooth to the touch. The bicellular hairs are too small and too soft to be perceptible.

D. *Second example: Inbred Yellow Dent No. L317.*—

Our second example is a yellow dent corn inbred, widely used in the United States corn belt, L317 (Cal. Tech. experimental field, Arcadia, summer 1948). The dermogram of this variety (fig. 3) differs from the preceding one chiefly by the outer face of the sheath (base of II). There the cushion hairs are very scarce and localized near the sheath lobes (these lobes are here much more developed than in the previous example). The cushion of these hairs is small, feebly protuberant, and not colored purple. The elements noticeable on the outer face of the sheath are ordinary hairs (P), spicules (P_1), bicellular hairs (B), cork and silica cells, the latter chiefly on the veins. The fundamental cells have thick, undulated walls (l_{3r}).

The inner face of the blade (summit of I, fig. 3) also shows some differences from the first example. Numerous big spicules (P_2) are borne on the principal ribs; on the bulliform stripes (C) cushion hairs are present, with an average density of 36 per square centimeter, but their cushion is very small compared to those of the first variety. On the outer face of the blade (summit of II, fig. 3) the cushion hairs are absent, but ordinary unicellular hairs can be noticed on the edges and on the auricular triangle. Bicellular hairs are present on the stripes between the veins, silica cells chiefly on the veins, and cork cells on both.

CONCLUSION

We have demonstrated for maize how it is possible to describe epidermic characters of cereals in order to use them in genetic and taxonomic studies.

1. When studying the epidermis of a cereal or a grass, the first step is to recognize its *structural characters*, i. e., the nature and the shape of its component cells. The shape of the silica cells (*S*), the presence and the shape of bicellular hairs (*B*), of cushion hairs (P_c), of bulliform cells (*C*), etc., must be specially investigated (see fig. 1). These structural characters give immediately the means to determine the general systematic position of the plant, i. e., the subfamily, tribe, to which it belongs. Each genus has a definite set of epidermis cells with well-defined shapes, as one of its fundamental characters. In all the species of a genus we find the same types of cells with exactly the same shapes, but they are differently distributed from one species to another.

2. The second stage of the study is therefore to determine how these categories of cells are distributed on the leaves and on the floral bracts. We thus obtain a second category of characters: the *distributive characters*, which may enable us to identify the species. This distribution of epidermic elements can be expressed in schematic maps or "*dermograms*" (fig. 2). The "*dermotype*" of a plant, the sum of its epidermic characters, will be thus expressed: (1) by a detailed drawing of each category of cells forming its epidermis (fig. 1), showing the structural characters and defining the general systematic position of the genus (subfamily, tribe); (2) by a *dermogram* (figs. 2 and 3) indicating the distribution of each type of cells on the outer and on the inner face of the leaf (the upper leaf being more characteristic, is preferably chosen), of the glume, lemma and palea. These dermograms show the distributive characters and give a means for typifying the species and varieties.

An interesting development in the genetic study of maize will be to identify the genes controlling the transmission of distributive epidermic characters, to analyze their linkage with other characters, and to find their positions in the chromosomes.

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